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Comparative Effects of Continuous and Interval Aerobic Training on Cellular Respiration Indices (OCR) and ATP Content in Skeletal Muscle of Rats with Type 2 Diabetes

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1. Round 1

1.1 Reviewer 1

Reviewer:

The paragraph beginning with *"Mitochondrial function depends on coordinated electron transport, oxygen consumption, oxidative phosphorylation, and ATP synthesis"* introduces OCR parameters but does not adequately explain the physiological significance of each index. The authors should provide a mechanistic justification for selecting basal respiration, ATP-linked respiration, maximal respiration, spare respiratory capacity, proton leak, and coupling efficiency as primary outcomes.

In the final paragraph of the Introduction, the authors hypothesize that interval training would produce stronger adaptations than continuous training. However, this hypothesis is not sufficiently justified. The authors should expand the discussion of previous comparative studies and explain why interval exercise is expected to exert superior effects specifically on mitochondrial respiration rather than merely on glycemic control.

In the Study Design section, the manuscript states that the research examined the effects of metabolic condition and exercise condition, yet no sample size calculation or power analysis is reported. Given the relatively small number of animals per group (n = 8), the authors should provide an a priori power analysis demonstrating that the study was adequately powered to detect biologically meaningful differences.

In the paragraph describing animal allocation, the sentence *"animals were randomly allocated to six groups"* lacks methodological detail. The manuscript should explicitly describe the randomization procedure, including whether simple randomization, block randomization, or computerized allocation was used. Such information is essential for assessing selection bias.

The section entitled *"Induction of Type 2 Diabetes"* raises concerns regarding the use of a single intraperitoneal STZ dose of 100 mg/kg. This dosage appears substantially higher than that commonly employed in high-fat diet/STZ models of type 2 diabetes and may induce extensive β-cell destruction more characteristic of type 1 diabetes. The authors should justify this dosage with references and discuss its compatibility with a type 2 diabetic phenotype.

Table 1 presents baseline and metabolic characteristics; however, no statistical comparisons are reported. It is unclear whether the differences in body weight, fasting glucose, and food intake reached statistical significance. The authors should include p-values and indicate which group comparisons were statistically significant.

In Table 2, OCR values are reported without units. The accompanying note explicitly states that *"OCR units and normalization method were not specified."* This issue is fundamental because OCR values cannot be interpreted without normalization parameters such as pmol O_2/min/mg tissue, per mitochondrial protein, or per cell count. These details must be included.

Authors revised the manuscript and uploaded the updated document.

1.2 Reviewer 2

Reviewer:

The paragraph reporting that animals with fasting glucose concentrations above 13.8 mmol/L were considered diabetic lacks additional metabolic characterization. To validate the diabetic model, measurements such as insulin concentrations, HOMA-IR, glucose tolerance testing, or HbA1c should be included. Without these parameters, the classification of the model as type 2 diabetes remains insufficiently substantiated.

In the Continuous Aerobic Training Protocol, the authors indicate that exercise intensity progressed from approximately 55% to 75% of aerobic capacity. However, no method is provided for determining aerobic capacity in rats. The manuscript should explain how exercise intensity was quantified and whether maximal running tests, lactate thresholds, or VO_2-related estimations were employed.

The paragraph describing the Interval Aerobic Training Protocol states that treadmill inclination was set at *"approximately 20–25 degrees."* This is a substantial incline that dramatically increases exercise intensity. The authors should justify the selection of this inclination and discuss its potential influence on mitochondrial adaptations independent of interval structure.

The Tissue Collection section indicates that samples were obtained 48 hours after the final exercise session. The authors should explain why this recovery period was selected and discuss whether it was intended to minimize acute exercise effects while preserving chronic training adaptations.

The most critical methodological limitation appears in the paragraph stating: *"Because the source manuscript did not specify the respiration platform, normalization strategy, ATP kit manufacturer, or measurement units, these methodological details must be completed."* This omission severely compromises reproducibility. The manuscript cannot be adequately

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evaluated without detailed information regarding the OCR measurement system, sample preparation procedures, calibration methods, normalization approach, and ATP assay specifications.

The paragraph describing mitochondrial respiration measurements does not specify whether OCR was assessed in isolated mitochondria, permeabilized fibers, intact cells, or tissue homogenates. Since these methodologies yield substantially different physiological interpretations, the authors must clearly identify the experimental preparation and provide a complete analytical protocol.

In the Statistical Analysis section, the authors report the use of two-way ANOVA but do not provide actual F-values, degrees of freedom, effect sizes, or interaction statistics. The manuscript should present complete ANOVA results to allow readers to assess the magnitude and significance of the reported effects.

Authors revised the manuscript and uploaded the updated document.

2. Revised

Editor's decision after revisions: Accepted.

Editor in Chief's decision: Accepted.

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